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A. Soil Studies—Desert Microflora. XII. Abundance of Microflora in Soil Samples from the Chile Atacama Desert

SECTION 12

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XVII. Bioscience

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1. Introduction

Prior to detection and characterization of possible extraterrestrial microorganisms, especially for Mars, preliminary investigations are being undertaken in various terrestrial desert areas.[1] Information about terrestrial desert environments, especially soils, microflora, and their relationships, can provide useful background information for design and instrumentation of life detection experiments. For this purpose, soil samples have been obtained from a number of desert regions, including the Chile Atacama Desert, known to be one of two most arid regions on this planet (the other is the Tanezrouft, in the Sahara Desert, Ref. 1).

[1]Cameron, R. E., "Areas for Soil Studies," Unpublished manuscript, JPL Biology Group, September 13, 1961.

2. Prior Investigations

Samples obtained from the Atacama Desert were collected from a completely barren, extremely arid area near Uriba, Chile (Fig. 1).[2] By means of aseptic technique, three samples were taken from approximately the surface 1-cm (No. 290), 1- to 4-cm level (No. 291), and from the 4- to 12-cm depth (No. 292).[3] The soils were sandy, with some white, crusty, aggregated salty material evident, especially with an increase in depth of soil (Fig. 2). The soil most closely resembles a Solonchak or azonal aridisol (Ref. 2).

Physical, chemical, and some microbiotic properties of the soils were presented in *SPS 37-35*, Vol. IV, pp. 214–222. Analyses of the samples showed that for all samples the bulk density was less than 1.0 g/cm^3, porosity was less than 67.0%, the pH of saturated paste was 7.7, electrical conductivity of the 1:5 soil: H_2O extract was between 325–2225 $\times$ 10^6 mho/cm at 25°C, and cation exchange capacity was 2.5 to 5.0 meq/100 g soil, with a significant

[2]Photographs taken by Father German Saa, Universidad del Norte, Antofagasta, Chile.

[3]Soils collected by R. W. Davies, Manager, Mission Definition, JPL Advanced Studies Section.

Fig. 1. Barren, eroded, wind-rippled soil and hills near Uriba, Chile Atacama Desert

Fig. 2. White, crusty, powdery and granular gypsum in extremely dry, sandy soil at sampling site near Uriba, Chile Atacama Desert

amount of the exchange complex occupied by cations Ca^{++}, Na^{+}, Mg^{++}, K^{+}, and anions $SO_4^{=}$ and Cl^{-}. The total organic matter content for all three samples was = 0.07%, the total C was = 0.10%, and the total N was = 0.01%. C content was either organic or inorganic, but N was entirely organic. All of the "routine" microbiological determinations indicated plate count values for aerobic and anaerobic bacteria, fungi, and algae which were either

Table 1. Plate counts on trypticase soy agar

Aerobes: bacteria + actinomycetes/soil

Soil No.	Psychrophiles (+5°C)	Mesophiles (+20°C)	Thermophiles (+55°C)[a]
290	3.5	8.0	0
291	80	4.0	0
292	14	38	0

[a]Not previously reported.

nil or extremely low (Table 1), as compared to values obtained for western U.S. desert soils (Ref. 3). However, growth obtained in dilution tubes of fluid thioglycollate medium indicated the presence of microflora of 10^6 to 10^7/g soil. Direct counts of microorganisms also substantiated the presence of a higher abundance of bacteria than indicated by plate counts using trypticase soy agar.[4]

Further cultures of these soils were attempted by dilution plates of a yeast extract/soil extract medium; it was then shown that higher counts could be obtained for all soils.[5] This medium had been previously shown to support a substantial growth of indigenous desert soil microflora, as well as *Bacillus subtilus*, following the introduction of a small inocculum into soil extract (*SPS 37-32*, Vol. IV, pp. 202–208). Plate counts obtained with trypticase soy agar or trypticase soy agar + soil extract for six other soil samples from the Atacama Desert near Antofagasta also indicated negligible or very low counts.[6]

Upon consideration of the extreme aridity of the Chile Atacama Desert, the unique properties of the soil samples, especially the apparent low abundance of microflora obtained by plate counts and relevance of the results to life detection experimentation, it was decided that further research would be conducted on these soils. This report will present results of bacteria + actinomycete abundance obtained with selective media, particularly with regard to chemoautotrophes.

3. *Materials and Methods*

Portions (1 g) of sieved ($\lesssim$2 mm), air-dry samples of each soil were put in 9 cm^3 of water and then placed on

[4]Weston, C. R., Monthly Progress Report, JPL Contract No. 1951321, Department of Biology, University of Rochester, College of Arts and Science, River Campus Station, Rochester, N. Y., November 15, 1965.

[5]Weston, C. R., Monthly Progress Report, *ibid* December 15, 1965.

[6]Opfell, J., "Effect of Soil Extracts in Medium in Detecting Aerobic Microbial Populations in Desert Soils from the State of Antofagasta, Chile," Aeronutronic Division, Ford Motor Co., Newport Beach, Calif., July 26, 1965 (unpublished report).

a wrist-action shaker for 10 min. Following completion of the suspension and dispersion of each sample, a 1-cm^3 portion of the soil suspension was then diluted serially by 1/10 and appropriate aliquots pipetted into sterile, plastic disposable petri dishes. Synthetic, nonsynthetic, and differential semisolid media were then poured into each petri dish and the recommended standard procedure followed for shaking plates to obtain colonies of soil microorganisms (Ref. 4). All incubations were carried out at room temperature (22°C), except for the carbon nutrition medium (Table 2, No. 3) which was incubated at 35°C. Plate counts were made as soon as colonies were macroscopically visible or could be distinguished with a Quebec Colony Counter equipped with a magnifier. For all cultures, the incubation period was terminated after 30 days, when no increase in colony abundance could be determined. All incubations were conducted between January 5 and March 2, 1966.

Most of the media used for this experiment were prepared according to standard procedures, and are recommended for various chemoautotrophic, heterotrophic, aerobic, anaerobic, and microaerophilic bacteria or actinomycetes (streptomycetes). The name of each medium and a reference to its preparation and composition is indicated in Table 3. The composition of media which are not included in any prior publication are given in Table 2.

Table 2. Composition of media prepared for Chile soils

Organic nutrition agar (No. 1)	
Trypticase soy broth	15.0 g
Yeast extract	2.0 g
Agar	15.0 g
Soil extract (Mohave soil No. 76-1)	400 cm^3
Tap water	600 cm^3
pH adjusted with 0.1N NaOH to 7.6	

Modified carbon nutrition agar (Refs. 5 and 6) (No. 3)	
Unbleached chitin power	10.0 g
KH_2PO_4	0.2 g
K_2HPO_4	0.8 g
$(NH_4)_2SO_4$	0.5 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$CaCl_2 \cdot 2H_2O$	0.01 g
Agar	15.0 g
Micronutrient solution (Ref. 7)	0.5 cm^3
Distilled H_2O	1000 cm^3
pH	7.0

Tap water agar[a] (No. 7)	
Si	26.6 ppm
Ca	54.2 ppm
Fe	0.09 ppm
Mg	15.0 ppm
Na + K	22.8 ppm
$CO_3^=$	0.0 ppm
HCO_3	242 ppm
$SO_4^=$	31.4 ppm
Cl	13.0 ppm
F	0.66 ppm
$PO_4^{\equiv}$	—
CO_2 (dissolved)	7.6 ppm
NH_3^+ (free)	0.01 ppm
N (organic)	0.15 ppm
NO_2	0.0 ppm
NO_3	0.43 ppm
Total solids	281 ppm
Total alkalinity	199 ppm
Total hardness	200 ppm
Agar	15.0 g
Tap H_2O	1000cm^3
pH	7.8

Neutralized acid soil extract agar (No. 8)	
Chile soil (appropriate soil No.), 25 g	
Extract with 100 cm^3 of 0.1N H_2SO_4	
Neutralize with 1.0N NaOH to pH 7	
Agar	15.0 g
Tap H_2O, q.s. to 1000 cm^3	

Neutralized alkaline soil extract agar (No. 9)	
Chile soil (appropriate soil No.), 25 g	
Extract with 100 cm^3 of 0.1N NaOH	
Neutralize with 1.0N H_2SO_4 to pH 7	
Agar	15.0 g
Tap H_2O, q.s. to 1000 cm^3	

Salt agar (No. 10)	
$CaSO_4$	0.3 g
NaCl	0.3 g
$MgCO_3$	0.1 g
KNO_3	0.1 g
$(NH_4)_2HPO_4$	0.1 g
$FeCl_3$	0.01 g
Agar	15.0 g
Micronutrient solution (Ref. 7)	0.5 cm^3
Distilled H_2O	1000 cm^3
pH adjusted with 0.1N NaOH to 7.6	

Complex iron-sulfur agar (No. 12)[b]	
$FeSO_4 \cdot (NH_4)SO_4 \cdot 6H_2O$	0.5 g
Fe powder, reduced	2.5 g
Fe_2O_3	2.5 g
Ferric ammonium citrate (half green; half brown)	5.0 g
S powder	5.0 g
$Na_2S_2O_3$	5.0 g
$CaCl_2$	0.1 g
$CaCO_3$	0.1 g
K_2HPO_4	0.1 g
KNO_3	0.1 g
$MgSO_4 \cdot 7H_2O$	0.1 g
Agar	15.0 g
Soil extract (Mohave soil No. 76-1)	10.0 cm^3
Micronutrient solution (Ref. 7)	0.5 cm^3
Tap H_2O	1000 cm^3
pH adjusted with 0.1N NaOH to 7.2	

[a]Average analysis of JPL tap water. Information supplied by T. Ackerman, Purification Supervisor, Water Department, Pasadena, Calif.

[b]Supernatant solution filtered through Whatman No. 1 paper.

Table 3. Abundance of microflora on selective agar media

Soil No.	Bacteria + actinomycetes × 10/g soil	Medium No.	Medium reference	Microflora group: Heterotrophes	Chemoautotrophes	Bacteria	Actinomycetes	Aerobes	Microaerophiles	Anaerobes	Halophiles	Sulfur and thiosulfate bacteria	Iron bacteria	Nitrogen bacteria
290	3.5	(1) Organic nutrition	Table 2	√	√	√	√	√						
291	35													
292	13													
290	1.5	(2) Thornton's standardized	Ref. 4, p. 5	√	√	√	√	√						
291	36													
292	145													
290	7.0	(3) Modified carbon nutrition	Refs. 5, 6; Table 2	√	√	√	√	√						
291	14													
292	4.0													
290	145	(4) Jensen's streptomycete	Ref. 4, p. 9	√	√	√	√	√						
291	10,000													
292	1550													
290	3.5	(5) Fluid thioglycollate	Ref. 8, p. 128	√	√	√	√	√	√	√		√		
291	3.0													
292	4.5													
290	0	(6) Fluid thioglycollate in CO_2 atmosphere	Ref. 8, p. 128	√	√	√	√		√	√		√		
291	0													
292	0													
290	1.5	(7) Tap water	Table 2		√	√	√	√						
291	22													
292	1.5													
290	1230	(8) Neutralized acid soil extract	Table 2		√	√	√	√			√			
291	4100													
292	37,000													
290	56	(9) Neutralized alkaline soil extract	Table 2		√	√	√	√			√			
291	46													
292	39													
290	6100	(10) Salts + micronutrients	Table 2		√	√		√			√			
291	3550													
292	2350													
290	13	(11) Starkey's sulfur oxidation	Ref. 4, p. 23		√	√		√			√	√		
291	145													
292	42													
290	5.0	(12) Beijerinck's thiosulfate oxidation	Ref. 4, p. 25		√	√	√	√			√	√		
291	20													
292	14													
290	64	(13) Van Delden's sulfate reduction	Ref. 4, p. 31	√	√	√		√	√		√	√		
291	1150													
292	1340													
290	3.5	(14) Complex iron-sulfur	Table 2		√	√		√	√			√	√	
291	48													
292	11													
290	0	(15) Heterotrophic iron oxidation	Ref. 4, p. 39	√		√		√					√	
291	0													
292	0													
290	0	(16) Leathen's autotrophic iron oxidation	Ref. 5, p. 533											

Table 3. Abundance of microflora on selective agar media (cont'd)

Soil No.	Bacteria + actinomycetes × 10/g soil	Medium No.	Medium reference	Microflora group: Heterotrophes	Chemoautotrophes	Bacteria	Actinomycetes	Aerobes	Microaerophiles	Anaerobes	Halophiles	Sulfur and thiosulfate bacteria	Iron bacteria	Nitrogen bacteria
291	0				√	√		√					√	
292	0													
290	0.5	(17) Ammonification (urea)	Ref. 4, p. 39											
291	0.5			√	√	√		√						√
292	0													
290	5.0	(18) Burk's nitrogen fixation	Ref. 4, p. 47											
291	8.0				√	√		√						√
292	4.0													

4. Results and Discussion

Variable results were obtained for plate counts of microorganisms, depending upon the medium, as indicated in Tables 1 and 3. Plate counts ranged from 0 to 3.7×10^5/g soil. It could not be shown that any one of the three soils contained the highest numbers of colonies, although for 12 of the 18 media, higher counts were obtained for subsurface soils No. 291 and No. 292, rather than surface soil No. 290.

The highest counts of microorganisms were obtained for Jensen's streptomycete agar (No. 4), neutralized acid soil extract agar (No. 8), salts + micronutrients agar (No. 10), and Van Delden's sulfate reduction agar (No. 13). Although Jensen's medium is intended primarily for the promotion of streptomycetes, it also permitted the growth of a number of hard or soft discrete orange-pink or opalescent pin-point, discoid, or star-shaped bacterial colonies. Neutralized acid soil extract agar also contained mixtures of both bacteria and streptomycetes. Colonies were primarily discrete opalescent, star-shaped, discoid, and fimbriated or lacerated. A number of hard, discrete, chromogenic (pink or orange), pin-point colonies were also observed. The salt agar medium, which was synthesized on the basis of available ions in the soils, contained a preponderance of smooth discrete, chromogenic (pink, orange or light brown), pin-point colonies. This medium did not promote the growth of streptomycetes. Van Delden's sulfate reduction agar had a number of discrete, pin-point or small, smooth, opalescent or chromogenic colonies and no streptomycetes.

In general, none of the other growth media provided as good a response for abundance of organisms, although the variety of colony characteristics was approximately the same, with a preponderance of discrete chromogenic bacterial colonies. However, media with a high percentage of organics, e.g., medium No. 1, did not contain chromogenic colonies. This was also true when trypticase soy agar was used as a culture medium for these soils. In a number of cases, colonies appeared on some of the media but they were not observed in other media. For example, with Starkey's sulfur oxidation agar there appeared on the surface of the agar discrete, black, raised, reticulated and lacerated colonies, along with a variety of the usual pin-point or small discoid, or star-shaped chromogenic and opalescent bacterial colonies and a few streptomycetes (Fig. 3). A microscopic examination of a number of the colonies from various plates shows that the organisms are primarily gram negative short bacilli and/or large or small gram negative cocci.

Agar plates of fluid thioglycollate medium did not give a good response. This is a broad spectrum medium which has been used without agar to promote the growth of many desert soil organisms, including bacteria, streptomycetes, and fungi.[7] By the dilution tube method, growth was previously obtained for all three Chile soils at 10^6 to 10^7/g soil using fluid thioglycollate (without agar). As has been found repeatedly for growth of organisms from desert soils, more abundant growth is obtained with solution cultures rather than agar plate cultures. Placing of fluid thioglycollate agar plates in a CO_2 atmosphere gave negative results for all three Chile soils. Evidently no significant

[7]Desert Microflora Program, unpublished results, 1961 to date, and Vol. IV of the following issues of *SPS: 37-35*, pp. 214–222; *37-34*, pp. 193–202; *37-32*, pp. 212–214 and pp. 196–202.

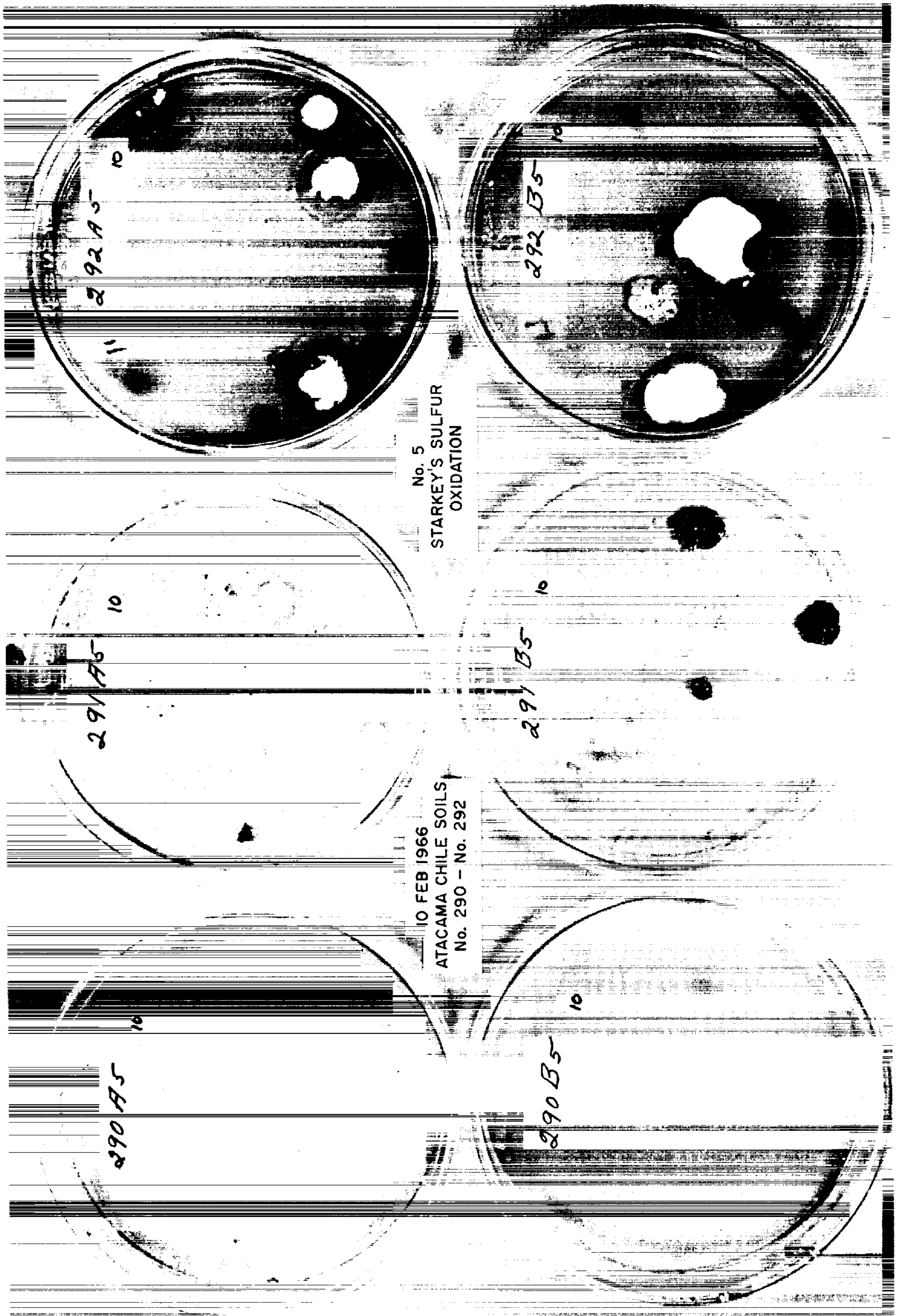

Fig. 3. Appearance of bacterial and streptomycete colonies isolated from subsurface soil No. 292 on Starkey's sulfur oxidation agar

numbers of anaerobes are present in these soils, as was indicated in *SPS 37-35*, Vol. IV.

Thornton's standardized medium is commonly used for obtaining a standard plate count of soil bacteria (Ref. 4). It contains organics (asparagine, mannitol, and soil extract) and promotes the growth of both heterotrophes and chemoautotrophes. However, for the Chile soils, it was not significantly better than an organic medium (No. 1) or tap water agar (No. 7).

When soils were first investigated for the Desert Microflora Program, a comparison was made between Thornton's standardized medium and trypticase soy agar for growth of microflora. It was found that for 28 soils from 5 sites, comparable results were obtained with the 2 media, or else counts were 1 dilution lower with Thornton's standardized medium. Incubation time was longer in some cases than with trypticase soy agar, which has therefore been used to determine the abundance of aerobic and anaerobic bacteria as well as streptomycetes in soils obtained from the Valley of 10,000 Smokes, Alaska (*SPS 37-32*, Vol. IV, pp. 196–202). In this case a medium was synthesized, based on properties of the soil and its external environment, e.g., sulfur fumes, and growth was obtained with the synthesized medium, whereas results were nil with trypticase soy agar. It would appear that for many desert soils trypticase soy agar is adequate, but where there are no macroplants the climate is extremely arid and soil properties are not typical; then a more inorganic medium should be used for culture purposes.

The salt medium (No. 10), which was synthesized on the basis of ionic composition of the Chile soils, provided a substantial increase in a number of colonies obtained, as compared to a number of the other media (Table 3). A comparison of this medium with Van Delden's sulfate agar shows that the sulfate ion is evidently important to the growth of organisms in Chile soils. This result is also consistent with the high percentage of granular or powdery gypsum present in these soils. Neither Starkey's sulfur oxidation medium (No. 11) nor Beijerinck's thiosulfate medium (No. 12) provided results as good. A complex iron sulfur medium (No. 14), which had been synthesized for Valley of 10,000 Smokes soils, also showed similar results.

One of the best media for growth, especially for subsurface soil No. 292, was a neutralized acid soil extract. The greatest abundance of organisms, 3.7×10^5/g soil, was obtained with this medium. The acid extraction procedure undoubtedly solubilized ions, such as sulfate or carbonate, which are present. Much lower results were obtained with the neutralized alkaline soil extract medium (No. 9).

Iron and nitrogen bacteria are not prevalent in the Chile soil samples. No growth was obtained for either heterotrophic or autotrophic iron oxidizers (media No. 15 and 16). A few ammonifiers or nitrogen fixers may be present (media No. 17 and 18).

Incubation times were generally long, as was found previously in *SPS 37-35*, Vol. IV. Colonies either were not evident or else reliable colony counts could usually not be obtained for 10 to 20 days. Growth periods were much faster with the synthesized salt medium and the neutralized acid soil extract medium, and discrete colonies, even though pin-point types, could be seen in several days. For most desert soils, such as those investigated in southwestern U.S., incubation times are commonly short, usually 8 to 48 hr. It has been found that it takes longer for colonies to be observed on routine culture media when desert soil properties, or the environment in which they occur, are not typical.[8]

5. Concluding Remarks

Abundance of microflora has been investigated for three soil samples from one site in the Chile Atacama Desert. Depending upon the nature of the selective medium, colony counts on agar plates ranged from 0 to 3.7×10^5/g soil. Four media provided the highest abundance of bacterial and streptomycete colonies. These were a synthesized salt medium, based on soil ions and salt content (No. 10), a neutralized acid soil extract made from each soil (No. 8), Jensen's streptomycete medium (No. 4), and Van Delden's sulfate medium (No. 13). Results were consistent with known environmental conditions and soil properties. Most of the bacterial colonies were discrete, hard or soft, chromogenic or opalescent, pin-point, or small and star-shaped. A large number of streptomycetes was also present, as indicated by growth on some of the media. Compared to results obtained on southwestern U.S. desert soils, there were more chromogenic colonies, very few heterotrophes, and a much longer incubation time was necessary before colonies could be observed and/or counted.

[8]Desert Microflora Program, unpublished results, 1961 to date.

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